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ON A CAUSE OF POSTOPERATIVE TRANSIENT
HYPOALBUMINEMIA
(INADEQUATENESS OF HOMOLOGOUS SERUM
ALBUMIN FOR THE HOST)

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Introduction

After a surgical operation accompanied by considerable bleeding, transient hypoalbuminemia is often found in spite of adequate blood transfusion. This shows that the loss of serum albumin by bleeding cannot be completely supplemented by blood transfusion.

Nutrient deficiency, leakage of protein through kidney and impaired protein anabolism in liver^{1),2),3),4)} are often shown after a large surgical operation, and these are generally considered as the main causes of the postoperative hypoalbuminemia.

However, according to our experimental and clinical studies, another factor unmentioned hitherto is the main cause of this transient hypoalbuminemia.

In the following, we will report the results of our studies on which we have based our opinion.

Transient hypoalbuminemia after surgical operation

In Table 1, we present the data of the serum protein of 31 patients on which lung surgery was done in our department⁵⁾. And this data shows that when bleeding exceeds 2000 cc, the serum protein remains low in spite of the appropriate transfusion.

Table 1. Total serum protein in 31 cases after surgery.

Bleeding Amount cc \ Serum Total Protein	decrease	no change	increase
Less than 500	1	3	2
500 ~1000	4	1	3
1000~2000	5	0	1
Over 2000	2	0	0

In most cases, this lowering of serum protein is observed from the first day after surgery and it reaches its lowest value at the 8th day and then gradually it attains the normal level in 4 weeks.

Table 2 illustrates the electrophoretical analysis of one patient who had been recorded 2000 cc of bleeding, and had received 2600 cc of whole blood transfusion.

Table 2. Serum protein fraction in the case after surgery.

	T.P. g/dl	relative value						absolute value (g/dl)				
		Al	α_1	α_2	β	γ	A/G	Al	α_1	α_2	β	γ
Pre-operative value	8.0	55.0	7.8	5.2	16.9	15.1	1.22	4.40	0.62	0.42	1.35	1.21
Immediately after operation	7.2	54.0	4.5	5.8	16.1	16.6	1.17	3.96	0.32	0.42	1.16	1.20
1 day after op.	7.4	53.8	5.2	6.1	11.5	23.4	1.16	3.98	0.39	0.45	0.85	1.73
3 days after	6.3	54.0	7.2	6.0	12.6	20.2	1.17	3.40	0.45	0.38	0.79	1.27
5 days after	6.7	56.1	6.7	5.8	15.9	22.0	1.27	3.69	0.45	0.39	1.07	1.47
1 week after	6.7	57.1	7.2	4.0	10.5	21.2	1.33	3.83	0.48	0.27	0.70	1.42
2 weeks after	8.2	56.0	6.7	6.0	11.7	20.6	1.27	4.59	0.55	0.49	0.96	1.69

Lobectomy : Bleeding 100 cc, Transfusion of whole blood 1600 cc

It can be seen from this table that lowering of total serum protein is due to the lowered absolute value of albumin.

Similar results were obtained in other cases where lowering of serum protein was seen.

But this finding is not a new one. Many authors reported that transient hypoproteinemia due to lowering of serum albumin was observed after surgery⁴⁾.

However, in those reports, it was thought that the main cause of hypoalbuminemia is not in bleeding, but in other trauma of surgery. We could

not agree easily to this suggestion. We saw many cases in which severe postoperative hypoalbuminemia was seen after severe bleeding even when the other surgical trauma was not so severe. Further, they consider that the above phenomenon is caused by lowering of albumin synthesis due to impairing of liver accompanied by bleeding. On the other hand, we think that the albumin in transfused homologous blood may have some differences in quality from autologous albumin remaining in the host, and as a result, the transfused homologous albumin can not be kept in the circulatory system.

The difference of transfusion effect between homologous and autologous albumin

Therefore the following experiment, using dogs, was designed to mimic the situation with passive bleeding and transfusion of the same amount of blood⁶⁾.

As a result, hypoalbuminemia was recognized in the group in which homologous blood transfusion after bleeding was done, but did not appear in the group in which autologous blood was replaced after bleeding without any procedure.

Then, in the next experiments, we prepared blood in which only the albumin was replaced by albumin of the other dog, while the other components were kept unchanged. We called this blood as Homologous albumin blood*¹.

Fig. 1 shows the average results of the total serum protein measurement of three groups, the control group of only passive bleeding, the autologous blood transfusion group and the homologous albumin blood transfusion group.

***¹ Supplement 1**

Two adult dogs, body weight over 10 Kg, were bled about 200 cc in A.C.D. solution. The sera were respectively separated from the blood, and crystalline ammonium sulfate (reagent grade) was slowly by added to each serum in proportion of half saturation in cold room (4°C). The mixture was centrifuged for 1 hr. at 21,000 rpm (Radius 8 cm). The supernatant fraction which we regarded as "albumin fraction" and precipitate which we regard as "globulin fraction" were dialyzed against saline several times. Albumin fraction from dog A and globulin fraction B were together added in solid portion of the blood of dog B. This mixture, namely artificial blood which we termed "Homologous albumin" was transfused into dog B. On the other hand, whole blood from dog C was used as auto-type blood for dog C.

Fig. 1. Total protein after transfusion of auto & homo type blood.

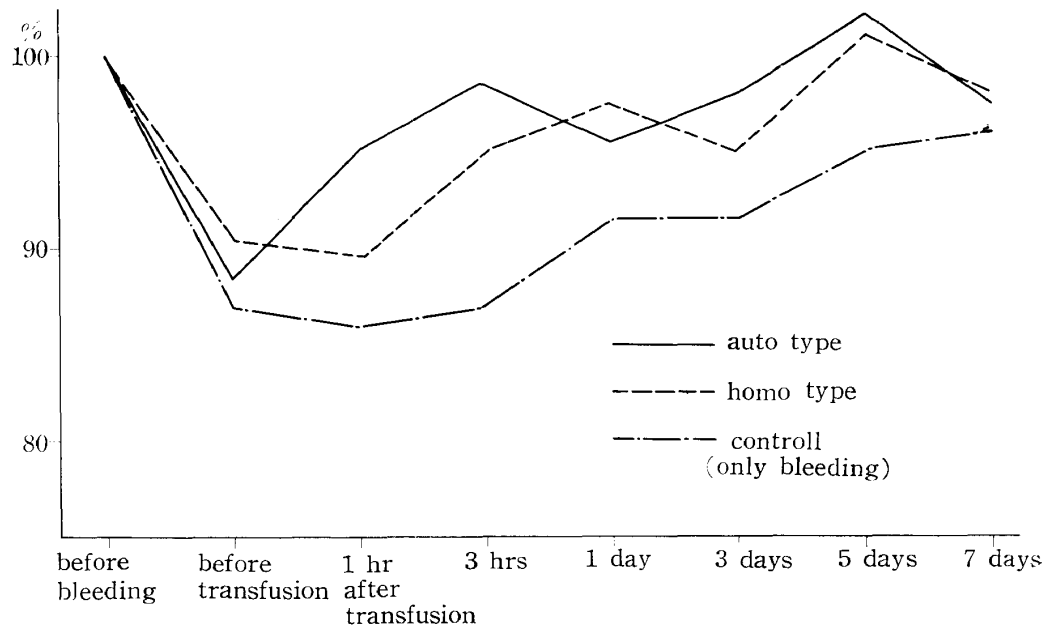
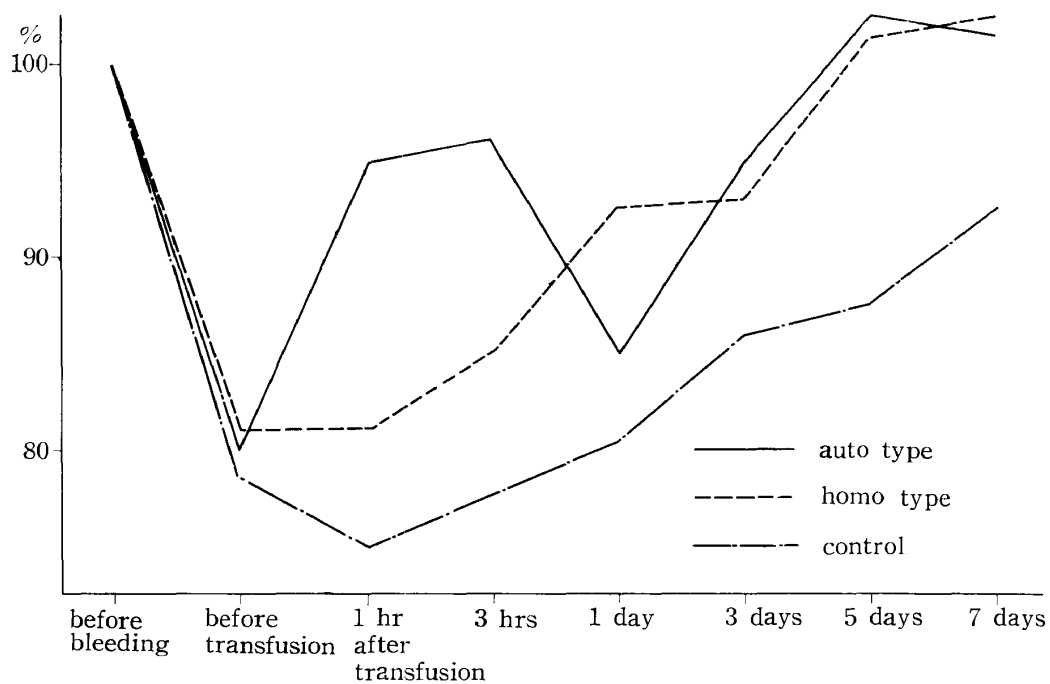


Fig. 2 shows the average results of the serum albumin measurement of the these groups.

Fig. 2. Serum albumin after transfusion of auto & homo type blood.



In these figures, the hypoalbuminemia is more conspicuous in the homo-type group than in the auto-type group.

Therefore, we have considered that the main cause of the hypoalbuminemia observed postoperatively is more related to the homologous blood transfusion than to the surgical trauma.

However, in the above experiments, the transfused albumin was measured without differentiating from the original albumin of the host. It could not be decided whether the hypoalbuminemia in our experiments was caused by decrease of the transfused albumin or not.

Trace of Transfused Albumin

Therefore, we used albumin labelled with FITC (Fluorescein isothiocyanate) in the following experiments so as to be able to trace the transfused albumin*².

Fig. 3 shows the value of serum protein after transfusion of homologous and autologous serum albumin in rabbits.

***² Supplement 2**

Approximately 60 ml of blood from each rabbit was allowed to clot at room temperature, then centrifuged and divided into Ca 30 ml serum, and 10.5 g reagentgrade (NH₄)₂SO₄ was slowly added to 30 ml of each serum, and the mixture was allowed to stand overnight at 4°C⁷, then centrifuged for 1 hr. at 21,000 rpm. The supernatant fraction comprised "albumin fraction" and was repeatedly dialyzed against phosphate buffered saline (0.85% NaCl-0.01 M Na-phosphate Buffer pH 6.8) at 4°C until the dialyzates no longer responded positively to a Nessler test. Ammonium sulfate must be removed completely before conjugation with FITC since it interferes with the reaction⁸.

To conjugate a serum with FITC, equal volume of carbonate bicarbonate buffer (pH 9.0 0.5 M freshly prepared) was added, solution stirred thoroughly at 4°C and then was titrated with same buffer to pH 9.0 approximately^{9),10),11)}.

One mg FITC per 40 mg of albumin was slowly and gradually added to buffered albumin solution and stirred efficiently over a period of about 8 hrs.

When conjugation was completed, the mixture was dialyzed with phosphate-buffered saline (0.85% NaCl Na-phosphate pH 6.8 0.01 M).

Then serum albumin solution conjugated with FITC was injected intravenously into autologous or homologous rabbit which had been previously bled.

Fig. 3. Total protein after albumin transfusion.

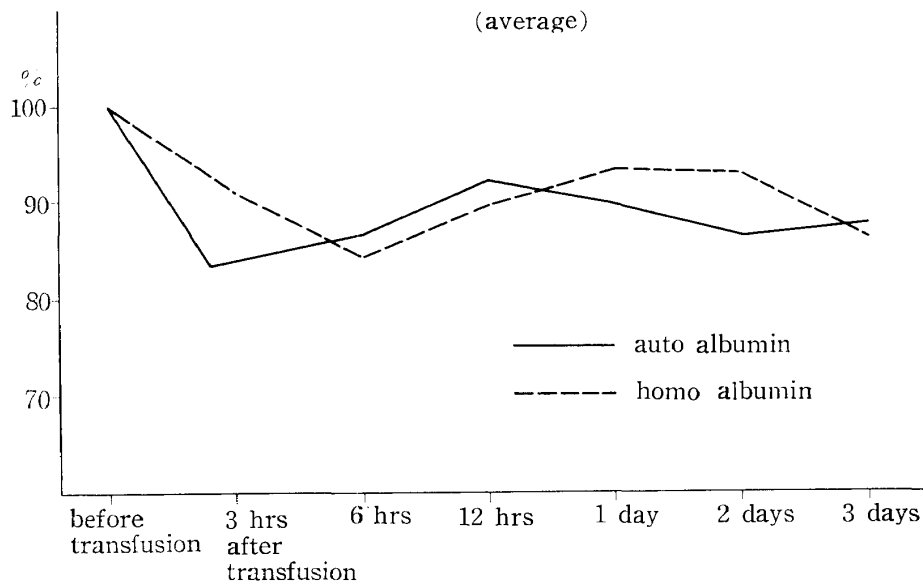
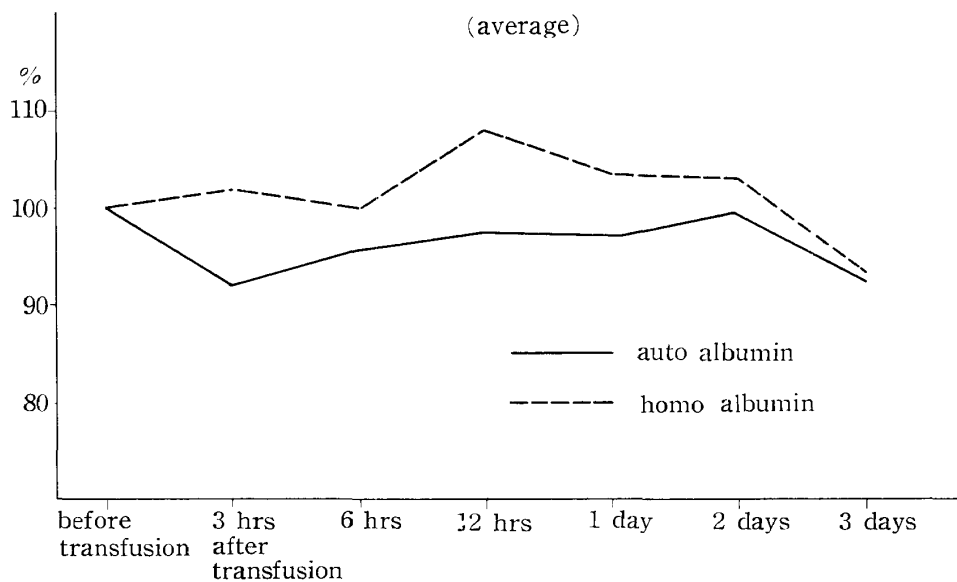


Fig. 4 illustrates the value of serum albumin after transfusion of autologous and homologous albumin individually. As is shown in this figure, serum albumin is less in the group of autologous albumin transfusion than in the group of homologous albumin, although buffered saline solution of only serum albumin without including the other components of blood was used.

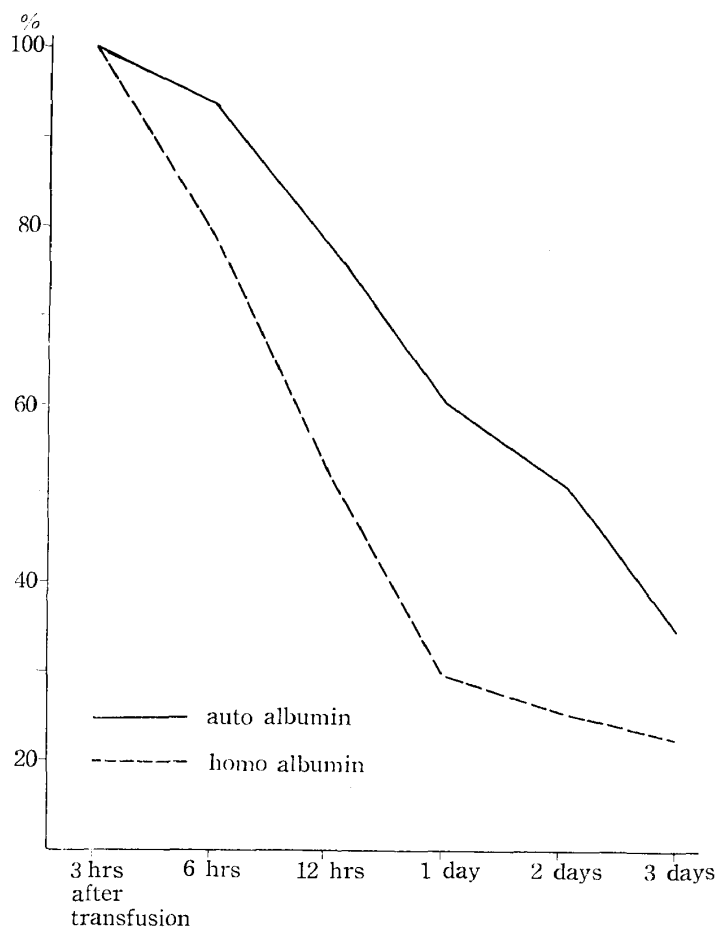
Fig. 4. Serum albumin after albumin transfusion.



These results may look contradictory to the former results, but the apparent contradiction will be solved by the next investigation.

Fig. 5 shows the disappearance rate of FITC from serum in the same experiment of Fig. 3 and Fig. 4. FITC disappears more rapidly in the group of homologous albumin transfusion than in the group of autologous albumin, and the decreasing rate in homo-shows always nearly half the value of that in auto-.

Fig. 5. Disappearance rate of FITC from serum.



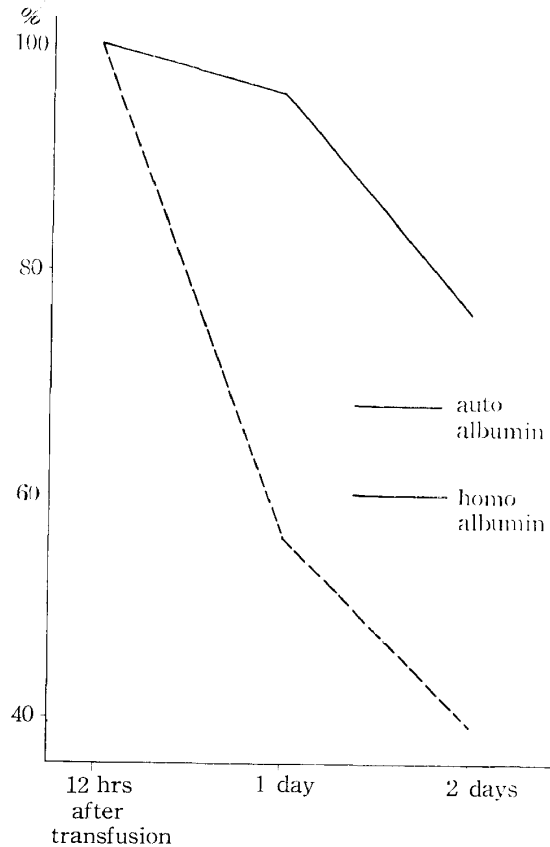
On the other hand, the rate of total albumin and FITC conjugated with albumin measured by the method described in supplement 3 is spotted in **Fig. 6***³.

*³ Supplement 3

Albumin fraction conjugated with FITC was obtained by boundary electrophoresis with starch.

Every protein fraction was eluted with saline 2 times, measured in accordance with method described by Folin-Ciocaltan and FITC conjugated with each albumin fraction was measured by spectrophotometer with 495 m μ .

Fig. 6. The ration of albumin conjugated with FITC & total albumin.



As shown in this figure, the amount of autologous serum albumin conjugated with dye does not show any remarkable change even on the second day after transfusion, and over 60% of homologous albumin with dye disappears from the circulating system on the second day.

From these results, we can presume that the greater part of homologous serum albumin has inadequate qualities for the host and is decomposed rapidly in the liver and other organs.

The degeneration engendered by the artificial procedures involved in the experimentation is only a part of the cause of rapid decomposition of homologous serum albumin, because autologous serum albumin subjected to a similar experimental procedure is not so much decomposed.

Subfractionation of serum albumin after surgery

Recently several methods for the subfractionation of serum albumin have been reported. Therefore we tried to make clear by the method of hydroxylapatite column chromatography^{13),14)} what subfraction decreases after trans-

fusion and what part is inadequate for the host.

By this method, we could subfractionate serum albumin to four subfractions, that is, Fr. I eluted by 0.04 M Na-phosphate buffer (pH 6.8) Fr. II by 0.07 M, Fr. III by 0.11 M and Fr. IV by 0.40 M.

Table 3 shows the normal value of every subfraction in the human. **Table 4** shows the value of subfractions of serum albumin in the human after surgery.

Table 3. Serum Albumin Subfraction in Human.

	Relative value				Absolute value g/dl				
	Fr I	Fr II	Fr III	Fr IV	Total Albumin	Fr I	Fr II	Fr III	Fr IV
No. 1	0.6	71.2	13.8	14.4	3.00	0.02	2.14	0.41	0.43
No. 2	5.4	55.1	15.1	24.4	4.80	0.26	2.65	0.73	1.17
No. 3	2.2	55.0	20.4	22.3	4.18	0.09	2.30	0.85	0.93
No. 4	3.0	53.3	4.0	37.7	4.02	0.12	2.15	0.16	1.51
No. 5	3.9	48.3	22.0	26.6	3.61	0.14	1.74	0.80	0.96
No. 6	12.2	39.8	10.0	38.0	4.35	0.53	1.73	0.44	1.65

Fr I Fraction eluted by 0.04 M phosphate Buffer (pH 6.8) Operation temperature 4°C
Fr II " 0.07 M " Hydroxylapatite Column 10 cm
Fr III " 0.11 M " (diameter 1.5 cm)
Fr IV " 0.40 M "

Table 4. The result of albumin subfractionation on a case after surgery.

Albumin	Relative value				Absolute value (g/dl)				
	Fr I	Fr II	Fr III	Fr IV	Total Albumin	Fr I	Fr II	Fr III	Fr IV
Pre-operative value	3.0	53.3	4.0	37.7	4.01	0.12	2.14	0.16	1.51
Immediately after operation	2.1	54.1	2.6	41.2	3.65	0.08	1.94	0.10	1.46
2 days after op.	16.8	53.5	3.2	26.5	3.39	0.57	1.81	0.11	0.90
4 days	17.8	37.8	1.2	43.2	2.48	0.44	0.94	0.03	1.07
8 days	7.4	40.9	10.6	41.2	2.88	0.21	1.18	0.31	1.19
15 days	3.9	40.4	8.8	47.3	3.15	0.12	1.27	0.28	1.49
30 days	3.4	63.5	4.9	28.0	3.59	0.12	2.28	0.18	1.01

Fr I Fraction eluted by 0.04 M Na-phosphate Buffer (pH 6.8) Operation temperature 4°C
Fr II " 0.07 M " Hydroxylapatite column 10 cm
Fr III " 0.11 M " (diameter 1.5 cm)
Fr IV " 0.40 M "

The amount of bleeding in this case is about 2000 cc and the amount of transfused blood is about 2600 cc. The change of serum protein fraction by electrophoresis in this case after surgery is supplementally shown in Table 5.

Table 6. The result of electrophoretical analysis on a case after surgery.

	T.P. g/dl	Relative value						Absolute value (g/dl)				
		Al	α_1	α_2	β	γ	A/G	Al	α_1	α_2	β	γ
Preoperative value	8.1	49.6	5.3	10.9	9.2	25.0	0.98	4.01	0.43	0.88	0.75	2.02
Immediately after operation	8.0	46.6	4.5	14.8	9.7	24.4	0.87	3.65	0.36	1.18	0.78	1.95
2 days after op.	6.9	49.1	2.8	8.9	9.8	29.4	0.96	3.39	0.29	0.61	0.68	2.03
4 days	6.0	41.4	4.0	12.1	14.5	28.0	0.71	2.48	0.24	0.73	0.87	1.68
8 days	6.9	41.7	11.0	18.6	9.8	18.2	0.72	2.88	0.76	1.28	0.68	1.26
15 days	7.6	41.5	5.7	11.4	15.3	26.1	0.71	3.15	0.43	0.87	1.16	1.98
30 days	7.7	46.6	4.6	8.5	14.3	26.0	0.87	3.59	0.35	0.65	1.10	2.00

Lobectomy : Bleeding 2000 cc, Blood Transfusion 2600 cc

As is shown in Table 4, decreasing of the amount of Fr. II suggests a most important role in post-operative hypoalbuminemia, although Fr. III and Fr. IV also decrease less in quantity compared with the progress of hypoalbuminemia.

In other words, it may be said that the inadequate part of serum albumin for other individuals is in Fr. II.

Conclusion

Finally we would like to report the following conclusion of our experiments.

(1) After a surgical operation accompanied by considerable bleeding, transient hypoalbuminemia is unavoidable even with adequate blood transfusion.

(2) The cause of post-operative transient hypoalbuminemia is due to the use of homologous blood.

Most of homologous serum albumin thus given is inadequate in quality for the host.

(3) The inadequate part of homologous serum albumin is in the sub-fraction Fr. II obtained by hydroxyl-appatite column chromatography.

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